Possible Influence of Cell Walls upon Ion Concentrations at Plasma Membrane Surfaces. Toward a Comprehensive View of Cell-Surface Electrical Effects upon Ion Uptake, Intoxication, and Amelioration[1]

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Plant uptake of ions, intoxication by ions, and the alleviation of intoxication by other ions often correlate poorly with ion concentrations in the rooting medium. By contrast, uptake, intoxication, and alleviation correlate well with ion concentrations at the plasma membrane (PM) surface computed as though the PM were bathed directly in the rooting medium with no effect from the cell wall (CW). According to two separate lines of analysis, a close association of CWs and PMs results in a slight increase in cation concentrations and a slight decrease in anion concentrations at the PM surface compared with concentrations when the CW is separated or has no effect. Although slightly different, the ion concentrations at the PM surface computed with and without close association with the CW are highly correlated. Altogether, the CW would appear to have a small effect upon ion uptake by the PM or upon intoxication or alleviation of intoxication originating at the PM surface. These analyses have been enabled by the recent evaluation of parameters required for the electrostatic models (Gouy-Chapman-Stern and Donnan-plus-binding) used to compute electrical potentials and ion concentrations in CWs and at PM surfaces.

Plant responses to ions in rooting media are long studied and currently active topics of investigation. Among the topics of interest are nutrition, intoxication, and the alleviation of intoxication. Each of the foregoing may involve the action of ions in cell apoplasts or in cell symplasts subsequent to transport across plasma membranes (PMs). Despite the possibility of a substantial influence of cell walls (CWs) upon PMs with regard to nutrition, intoxication, and the alleviation of intoxication, little is known about this possible influence. PMs and CWs are often studied in isolation with regard to plant-ion interactions. Examples include biophysical and genetic investigations of ion channels in PMs (Véry and Sentenac, 2003) and ion-exchange properties in CWs (Sattelmacher, 2001). Nevertheless, several topics are increasingly being studied in terms of CW-PM interactions. These include aluminum toxicity (Horst et al., 1999; Rengel and Zhang, 2003; Sivaguru et al., 2003) and some developmental phenomena (see the topical issue of Plant Molecular Biology, Vol. 47, Nos. 1 and 2 [2001]). Despite progress in some areas, fundamental questions about CW-PM interactions remain.

My interest is in the possible electrostatic interactions between CWs and PMs and whether such interactions affect ion concentrations at the PM surface and thereby affect nutrition, intoxication, and the alleviation of intoxication. A century-old electrostatic theory (Gouy-Chapman) has been used in the study of some membrane phenomena, especially photosynthesis (for review, see Barber, 1980), and is currently being used to interpret quantitatively the interactive effects of multiple ions (Kinraide, 1999, 2001, 2003a, 2003b; Zhang et al., 2001; Ahn et al., 2004). The quantitative nature of these studies has been enabled by the recent evaluation of parameter values needed in a Gouy-Chapman-Stern model for the determination of PM-surface electrical potentials (Yermiyahu et al., 1997; Kinraide et al., 1998; Vulkan et al., 2005; Table I). Knowledge of these potentials (illustrated in Fig. 1) then enables the computation of PM-surface ion activities (see below).

Figure 2 illustrates the uptake of selenium by wheat (*Triticum aestivum*) roots in response to selenate activity in rooting media that are variable in pH and in CaCl$_2$ and MgCl$_2$ concentrations. These solutes reduce PM-surface negativity and thereby increase the PM-surface activity of selenate. Clearly, uptake and intoxication (as noted in parallel studies) is more closely related to PM-surface activities than to activities in the rooting media, and the same is true for other ions (Zhang et al., 2001; Kinraide et al., 2004).

The studies just noted have treated the PMs of root cells as though the CWs had no influence upon the PM. The parameters for the electrostatic models used to compute ion activities at the PM surface were obtained from PM vesicles or cell protoplasts (Yermiyahu et al.,

Table I. *Parameter values for Gouy-Chapman-Stern and Donnan-plus-binding models for the computation of PM-surface potentials and CW electrical potentials*

The binding constants, exemplified by $K_{R,Ca}$ $(=[RCa^+]/([R^-][Ca^{2+}])$ and $K_{P,Ca}$ $(=[PCa^{2+}]/([P^0][Ca^{2+}])$, refer to the reactions $R^- + Ca^{2+} \rightleftarrows RCa^+$ and $P^0 + Ca^{2+} \rightleftarrows PCa^{2+}$. R^- is the surface density or concentration of negative charges. P^0 is the surface density of neutral binding sites at the PM surface. R_{Total} and P_{Total} are based upon the number of negative and neutral sites, respectively, in the absence of any bound ions. Concentrations of ions in the binding reactions (e.g. $[Ca^{2+}]$) refer to values at the PM or CW surfaces or in the CW, not the rooting medium. Constants for binding at neutral sites are 1/180 the value at the charged sites ($K_{P,I} = K_{R,I}/180$). (See Kinraide et al. [1998], Kinraide [2001], Shomer et al. [2003], and Vulkan et al. [2005] for more complete treatments of the electrostatic models.)

Model Parameters	G-C-S for PM	Donnan-Plus-Binding for CW	G-C-S for CW
R_{Total}	0.307 μmol m^{-2}	0.0211 M	0.0590 μmol m^{-2}
P_{Total}	2.4 μmol m^{-2}	0 M	0 M
$K_{R,Na}$, $K_{R,K}$	1 M^{-1}	0 M^{-1}	0 M^{-1}
$K_{R,Zn}$	5	0	0
$K_{R,Ca}$, $K_{R,Mg}$	30	0	0
$K_{R,Cu}$	400	0	0
$K_{R,La}$	2,200	0	0
$K_{R,Al}$	20,000	0	0
$K_{R,H}$	21,500	9,450	1,830

1997; citations in Kinraide et al., 1998) and have then been applied as though the CW were not present. Despite the fact that the question "What about the CW?" is often heard, few answers have emerged.

In this study, I shall present an analysis of possible CW effects upon ion concentrations at the PM surface. Specifically, I shall consider the differences in PM-surface ion concentrations when the PM is bathed directly in the rooting medium without effect from the CW and when the PM and the CW are intimately associated (Fig. 1).

COMPUTATION OF ION CONCENTRATIONS AT PM SURFACES

Ion Concentrations at PM Surfaces Bathed Directly in the Rooting Medium

When plant-root PMs contact a solution, the Müller equation (expressing the Gouy-Chapman theory) describes the relationships among the PM-surface charge density (σ_{PM}), the ion concentrations in the solution ($[I^Z]$), and the electrical potential at the PM surface (ψ_{PM}) (Barber, 1980; Tatulian, 1999). In the equation that follows, I use double subscripts to designate an interaction between the PM surface and the rooting medium; thus, $\sigma_{PM,medium}$ designates the surface charge density when the PM is bathed in the medium, and $\psi_{PM,medium}$ designates the electrical potential when the PM is bathed in the medium (actually, $\psi_{PM,medium}$ is the potential difference $\psi_{PM} - \psi_{medium}$).

$$\sigma^2_{PM,medium} = 2\varepsilon_r\varepsilon_0 RT\Sigma_i[I^Z]_{medium}$$
$$\times (\exp[-Z_iF\psi_{PM,medium}/(RT)] - 1), \quad (1)$$

where $2\varepsilon_r\varepsilon_0 RT = 0.00345$ at 25°C for concentrations expressed in M, and $F/(RT) = 1/25.7$ at 25°C for ψ expressed in mV. ε_r is the dielectric constant for water;

ε_0 is the permittivity of a vacuum; and F, R, and T are the Faraday constant, the gas constant, and the temperature, respectively.

$\sigma_{PM,medium}$, expressed in Coul m^{-2}, is not constant. It is a function of the surface charges contributed by the usually negatively charged structural components of the PM itself (e.g. $-COO^-$, $-OPO_3H^-$, $-NH_4^+$, etc.) and of the ions that bind to the PM surface (e.g. $-COOAl^{2+}$, $-OPO_3HCa^+$, $-NH_4Cl^0$, etc.). Ordinarily, the PM surface is negative, but adequate concentrations of strongly binding cations (e.g. H^+ and Al^{3+} in acidic soils) may convert the PM surface from negative to positive. To compute $\sigma_{PM,medium}$, one must know the density of charged structural components at the PM surface and the characteristics of ion binding to the PM surface (e.g. equilibrium constants for binding at both charged and neutral sites; Table I). Eventually, a surface charge density R^- (in mol unit charge m^{-2}) is obtained, which when multiplied by F (in Coul mol^{-1}) yields $\sigma_{PM,medium}$ (in Coul m^{-2}).

Recent years have witnessed much progress in obtaining parameter values needed for the use of the Müller equation (Table I) to find $\psi_{PM,medium}$ by an iterative process described in Kinraide et al. (1998), and computer programs are available from the author. Thus, $\psi_{PM,medium}$ may be computed for many solutions. With the availability of $\psi_{PM,medium}$, the concentration of ions at the PM surface may be computed from a Boltzmann equation.

$$[I^Z]_{PM,medium} = [I^Z]_{medium} \exp[-Z_iF\psi_{PM,medium}/(RT)] \quad (2)$$

$[I^Z]_{PM,medium}$ signifies the concentration when the PM is in direct contact with the rooting medium. Equation 2 implies the assumption that the CW, if present, has no influence.

Many readers, and physiologists in particular, may be more accustomed to a variation of Equation 2 known
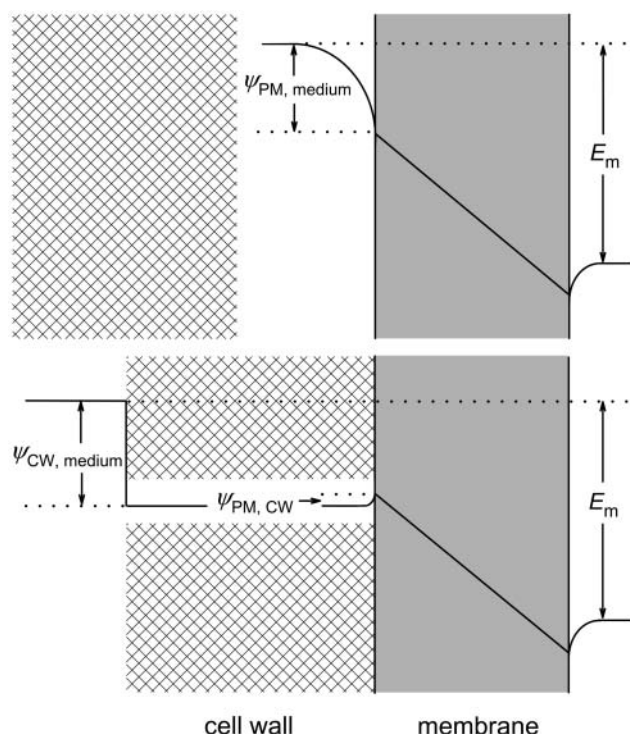
Figure 1. Possible electrostatic interactions between CWs and PMs. In the top portion of the figure, the PM is not influenced by the CW because of physical separation or some other reason. In the bottom portion, the PM is considered to be bathed by the Donnan-phase solution of the CW. E_m (-105 mV) is the transmembrane electrical potential difference from rooting medium to cell interior. $\psi_{PM,medium}$ (-41.8 mV) is the electrical potential of the PM surface when the PM is bathed directly by a rooting medium composed of 0.1 mM $CaCl_2$, 1 mM NaCl, and 0.001 mM $LaCl_3$ at pH 5.6 (solution no. 1 in Table II). $\psi_{CW,medium}$ (-50.3 mV) is the electrical potential of the CW. $\psi_{PM,CW}$ (5.1 mV) is the electrical potential of the PM surface relative to the CW. The PM surface-to-surface potential difference (indicated by the sloping lines) plays a role in ion transport (Kinraide, 2001) but is not considered in this study. Unlike the vertical dimensions (a voltage scale), the horizontal dimensions are not drawn to scale. Moreover, the depiction of the electrical profile is simplified as in the step change at the outer CW surface and in the straight lines through the CW and the PM.

as the Nernst equation, which incorporates chemical activities rather than concentrations (Nobel, 1991). If activity is the appropriate measure for the electrochemical equilibrium of an ion and if the electrical potential is accurately expressed as $\psi_{PM,medium}$, then the following equation will define the activity of ions at the PM surface because ion activity in a bulk-phase medium can be computed accurately.

$$\{I^Z\}_{PM,medium} = \{I^Z\}_{medium} \exp[-Z_i F \psi_{PM,medium}/(RT)]$$
(3)

The problem is that the derivation of the Müller equation incorporates Equation 2, not 3. Consequently, Equations 2 and 3 are both correct only if the activity coefficients remain constant at all distances from the

PM surface. This seems counterintuitive because the sum of free ion concentrations (and thus, presumably, the ionic strength from which activity coefficients are computed) can be much greater at the PM surface. In previous studies, I have assumed the constancy of activity coefficients and have related ion uptake and toxicities to $\{I^Z\}_{PM,medium}$ rather than to $[I^Z]_{PM,medium}$, but the latter is usually a good indicator of physiological response, and for the remainder of this presentation I shall use concentrations principally.

$[I^Z]_{PM,medium}$ appears to influence ion uptake and ion toxicity much more directly than $[I^Z]_{medium}$; that is, the former correlates with uptake and toxicity much more strongly than the latter (citations above). Nevertheless, these strong correlations are only weak evidence that $[I^Z]_{PM}$ has been computed correctly. Implicit in the
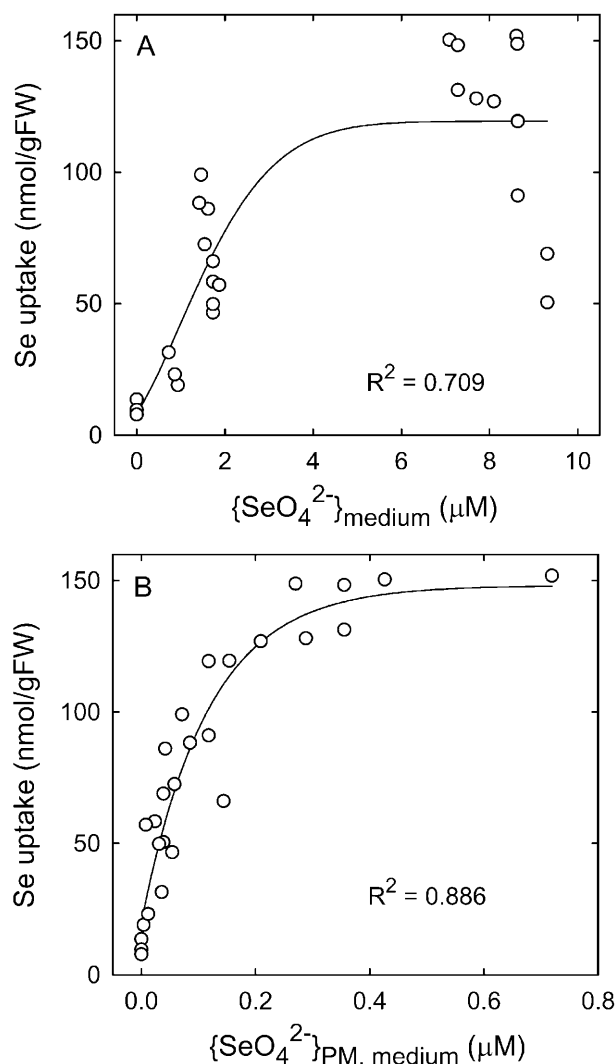
Figure 2. Selenium uptake by roots in response to SeO_4^{2-} activities in the rooting medium and at the PM surface. Atlas 66 wheat seedlings were cultured in media variously supplemented with $CaCl_2$ and $MgCl_2$, adjusted to several pH values. This figure is redrawn from Kinraide (2003a).

preceding treatment is the assumption that the PM is bathed in the rooting medium directly or that the CW has no influence on $[I^Z]_{PM}$. As an alternative, I shall assume an intimate interaction between CW and PM—namely, that the PM is bathed not by the rooting medium but by the solution in the CW Donnan phase.

## Ion Concentration at PM Surfaces Bathed in the CW Donnan Phase

Measurements of the (nearly always negative) electrical potential of the CW Donnan phase ($\psi_{CW,medium}$) are problematical. In several studies microelectrodes were pushed against cell surfaces and the potentials were recorded as the composition of the bathing solutions was changed (Nagai and Kishimoto, 1964; Saftner and Raschke, 1981; Shomer et al., 2003). These measurements corresponded substantially with expectation in that the depolarizing effectiveness of ions was related to size and charge (Shomer et al., 2003), corresponded to the strength of adsorption by CWs as determined by chemical assays (Franco et al., 2002), was in agreement with ζ-potential responses of CW fragments (O'Shea et al., 1990), and corresponded roughly to the depolarizing effectiveness against PMs (Shomer et al., 2003).

A major difference between the PMs and the CWs is that the binding of ions to CWs appears to be much weaker than to PMs, especially with respect to apparent binding at neutral sites (Table I). In fact, all of the values in the "Donnan-Plus-Binding for CW" column in Table I appear to be small, except for the value for $K_{R,H}$. Chemical assays of isolated CW material indicate much greater values (references in Shomer et al., 2003), but the values presented in Table I were determined in vivo or at least in situ and pertain to sites that were accessible to measurements of electrical potential and available to reversible binding. If the reported values for $R_{Total} = 1$ M referred to sites available to reversible binding, then $\psi_{CW,medium} \approx -200$ mV in 0.1 mM NaCl. Such negative potentials have not been measured to my knowledge.

A Donnan-plus-binding model for the computation of $\psi_{CW,medium}$ was developed, and the success of this model for $\psi_{CW,medium}$ and the success of the Gouy-Chapman-Stern model for $\psi_{PM,medium}$ can be assessed from Figure 3. Assuming that $\psi_{CW,medium}$ can be computed, then ion concentrations in the CW Donnan phase can be computed from this equation, assuming once again that concentration is an adequate measure for electrochemical equilibrium.

$$[I^Z]_{CW,medium} = [I^Z]_{medium} \exp[-Z_i F \psi_{CW,medium}/(RT)] \quad (4)$$

Now it is possible to rewrite the Müller equation so that surface electrical potentials of PMs bathed in the CW Donnan phase may be calculated.
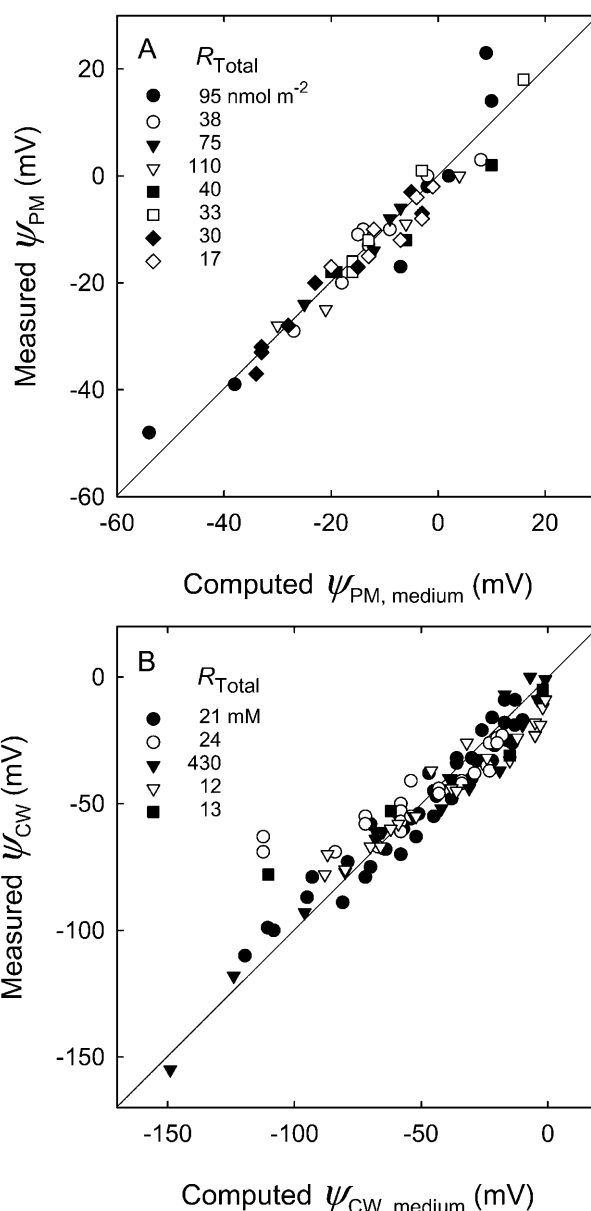
Figure 3. A comparison of studies in which $\psi_{PM,medium}$ and $\psi_{CW,medium}$ were measured and computed. A, Parameters for a Gouy-Chapman-Stern model were evaluated for eight studies; B, parameters for a Donnan-plus-binding model were evaluated for five studies. Optimized values for total negative sites (R_{Total}) were computed for each study, but a single suite of binding constants was evaluated for the pooled PM data and for the pooled CW data (see Table I). The figure is redrawn from Shomer et al. (2003).

$$\sigma^2_{PM,CW} = 2\varepsilon_r\varepsilon_0 RT\Sigma_i [I^Z]_{CW,medium}$$
$$\times (\exp[-Z_i F\psi_{PM,CW}/(RT)]-1) \quad (5)$$

$\psi_{PM,CW}$ expresses the potential difference $\psi_{PM} - \psi_{CW}$, and $\sigma_{PM,CW}$ is the surface charge density of PMs bathed in the CW Donnan phase. Finally, ion concentrations at the PM surface bathed in the CW Donnan phase may be computed from

Table II. *Some electrical potentials computed for solutions composed of three salts and adjusted for pH with HCl*

Concentrations are expressed in mM and potentials in mV.

No.	pH	$CaCl_2$	NaCl	$LaCl_3$	$\psi_{CW,medium}$	$\psi_{PM,CW}$	$\psi_{PM,medium}$
1	5.6	0.1	1	0.001	−50.3	5.1	−41.8
2	5.6	0.1	1	0.01	−45.4	17.1	−24.5
3	5.6	0.1	10	0.001	−21.6	−18.4	−37.9
4	5.6	0.1	10	0.01	−21.3	−3.6	−22.8
5	5.6	1.0	1	0.001	−27.9	−6.9	−32.2
6	5.6	1.0	1	0.01	−27.5	3.2	−21.7
7	5.6	1.0	10	0.001	−16.6	−15.3	−29.8
8	5.6	1.0	10	0.01	−16.5	−5.9	−20.4
9	4.6	0.1	1	0.001	−40.4	1.6	−33.9
10	4.6	0.1	1	0.01	−37.5	11.7	−21.8
11	4.6	0.1	10	0.001	−16.2	−14.2	−27.1
12	4.6	0.1	10	0.01	−16.1	−5.5	−18.9
13	4.6	1.0	1	0.001	−22.8	−6.0	−25.5
14	4.6	1.0	1	0.01	−22.5	0.9	−18.6
15	4.6	1.0	10	0.001	−12.8	−11.9	−22.1
16	4.6	1.0	10	0.01	−12.8	−6.3	−16.8

$$[I^Z]_{PM,CW} = [I^Z]_{CW,medium} \exp[-Z_i F \psi_{PM,CW}/(RT)]. \quad (6)$$

Because $[I^Z]_{medium} \exp[-Z_i F \psi_{CW,medium}/(RT)]$ from Equation 4 may be substituted for $[I^Z]_{CW,medium}$ in Equation 6, $[I^Z]_{PM,CW}$ may be computed from

$$[I^Z]_{PM,CW} = [I^Z]_{medium}$$
$$\times \exp[-Z_i F(\psi_{CW,medium} + \psi_{PM,CW})/(RT)]. \quad (7)$$

A Comparison of $[I^Z]_{PM,medium}$ and $[I^Z]_{PM,CW}$

If the CW has had no effect upon $[I^Z]$ at the PM surface, then $[I^Z]_{PM,CW} = [I^Z]_{PM,medium}$. That will be the case, of course, for neutral solutes ($Z = 0$) or for uncharged CWs. Although it is not readily apparent from the equations, if $\psi_{CW,medium}$ is negative and Z is positive, then $[I^Z]_{PM,CW} > [I^Z]_{PM,medium}$. That is, the model predicts that when the (nearly always negative) CW interacts with the PM, the effect is to cause (usually small) increases in cation concentrations and decreases in anion concentrations at the PM surface. Furthermore, $[I^Z]_{PM,CW}$ is approximately proportional to $[I^Z]_{PM,medium}$. I shall illustrate these features by an analysis of some solutions presented in Table II.

In solution number 1, all solutes are at a minimum relative to the other solutions so that $\psi_{CW,medium}$ (−50.3 mV) is more negative than elsewhere. When the 1 mM NaCl was increased 10-fold, as in solution number 3, the negativity of $\psi_{CW,medium}$ declined to −21.6 mV. Consequently, $[La^{3+}]_{CW,medium}$ was driven down to

Table III. *Concentrations of Na^+ and La^{3+} in CW and PM phases computed from the solutions presented in Table II*

Concentrations are expressed in μM.

No.	$[Na^+]_{CW,medium}$	$[Na^+]_{PM,CW}$	$[Na^+]_{PM,medium}$	$[La^{3+}]_{CW,medium}$	$[La^{3+}]_{PM,CW}$	$[La^{3+}]_{PM,medium}$
1	7,070	5,810	5,090	353.0	196.0	132.0
2	5,840	3,000	2,590	1,990.0	270.0	174.0
3	23,200	47,400	43,700	12.5	107.0	83.5
4	22,900	26,400	24,300	121.0	184.0	143.0
5	2,970	3,870	3,500	26.1	58.1	42.8
6	2,920	2,570	2,320	248.0	170.0	125.0
7	19,100	34,600	31,900	6.9	41.3	32.4
8	19,000	23,900	22,100	68.6	136.0	108.0
9	4,810	4,510	3,750	111.0	91.8	52.6
10	4,300	2,730	2,330	798.0	203.0	127.0
11	18,800	32,600	28,700	6.6	34.7	23.7
12	18,700	23,200	20,900	65.3	124.0	91.3
13	2,430	3,060	2,700	14.3	28.8	19.6
14	2,400	2,320	2,060	138.0	125.0	87.8
15	16,500	26,200	23,700	4.5	18.0	13.2
16	16,400	21,000	19,200	44.4	92.4	70.7

12.5 μM from 353 μM (Table III), and $[Ca^{2+}]_{CW,medium}$ and $[H^+]_{CW,medium}$ were driven down as well. These ions (La^{3+}, Ca^{2+}, and H^+) depolarize the PM strongly (and depolarize the PM more strongly than the CW [see model parameters in Table I]), so the declines in their concentrations more than offset the increase in $[Na^+]$ (from 5,810 μM to 47,400 μM) because Na^+ is a weakly depolarizing ion. The net effect is an increase in the negativity of $\psi_{PM,CW}$ from 5.1 mV to -18.4 mV. These opposing trends (declining negativity in $\psi_{CW,medium}$ and increasing negativity in $\psi_{PM,CW}$) cause Equations 2 and 7 to compute approximately similar values because the change in $\psi_{PM,medium}$ (from -41.8 mV to -37.9 mV) is comparable to the change in $\psi_{CW,medium} + \psi_{PM,CW}$ (from -45.2 mV to -40.0 mV). Thus, we see in Table III that the decline in $[La^{3+}]_{PM,CW}$ (45%) is not greatly different from the decline in $[La^{3+}]_{PM,medium}$ (37%).

Figure 4A presents a comparison of $[I^Z]_{PM,medium}$ and $[I^Z]_{PM,CW}$ for Ca^{2+}. $R^2 = 0.997$ and the slope is 1.21. R^2 values for La^{3+} and Na^+ are 0.970 and 0.999, respectively, and the slopes are 1.46 and 1.09, respectively. These trends meet expectations. R^2 is 1.000 for uncharged solutes and declines as charge increases; slope

equals 1 for uncharged solutes, increases as positive charge increases, and declines as negative charge increases. That is, the presence of the CW increases the PM concentration of cations and reduces the PM concentration of anions. If the CW were uncharged, then the R^2 and slope would be 1; that is, the concentration of cations at the PM surface would be the same with or without the wall.

Figure 4 presents additional computations relevant to physiological responses. PM-surface activities are not greatly different from PM-surface concentrations, at least in these solutions (Fig. 4D). Because PM-surface activities correlate well with physiological responses (Fig. 2B), PM-surface concentrations will too; CW concentrations (or activities) will correlate poorly (Fig. 4B); and concentrations (or activities) in the rooting medium will correlate poorly also (Fig. 4C).
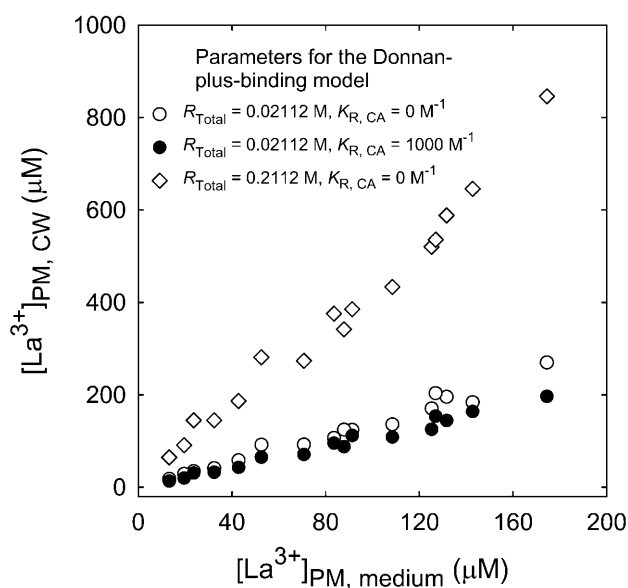
What if the CW were more highly charged than the 0.0211 (moles negative charge)/(liter CW volume) found to be optimum for the computation of $\psi_{CW,medium}$ (Table I)? This is a possibility because glass microelectrodes and ζ-potential measurements may not access directly the electrical potentials of the CW
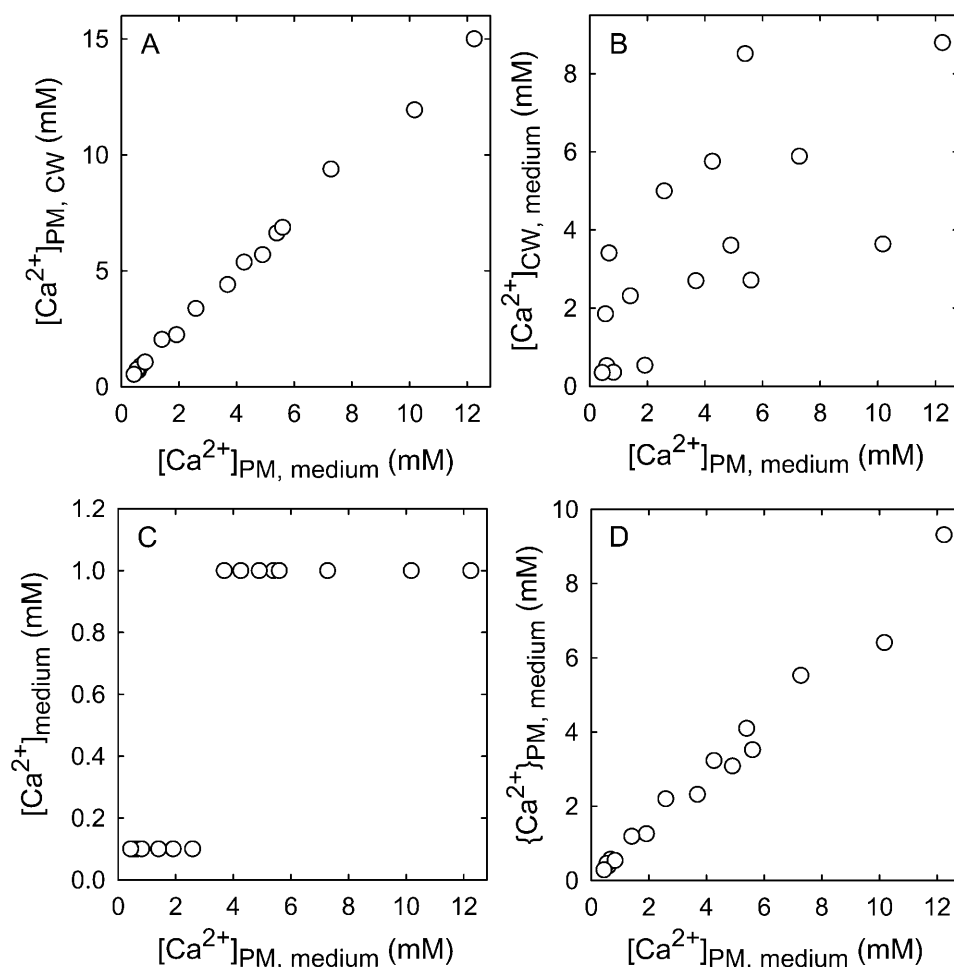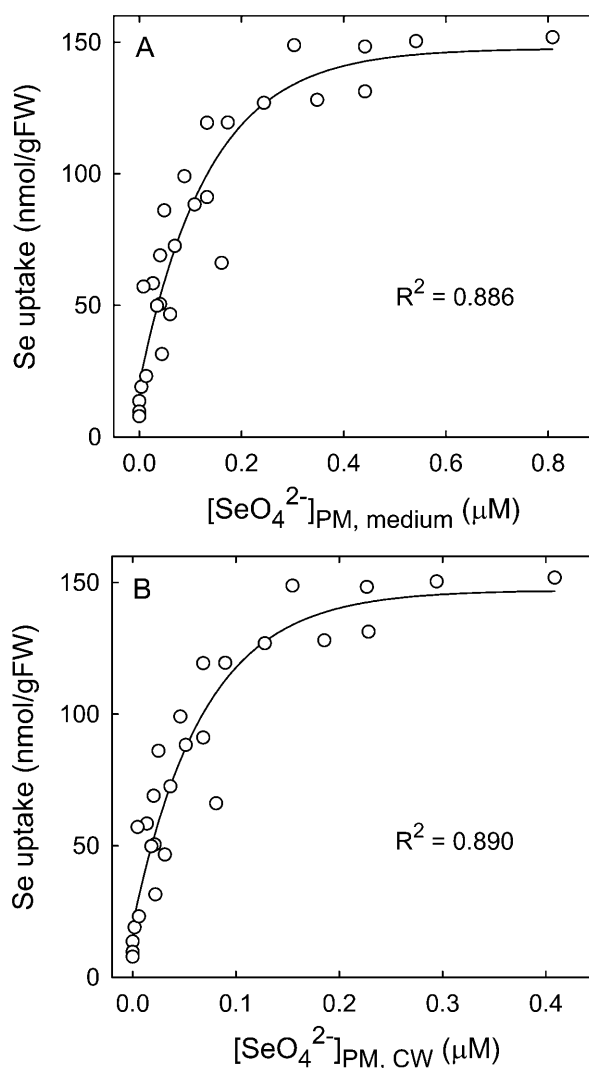

Figure 4. Plots of Ca^{2+} concentrations in various phases for cells bathed in the rooting media presented in Table II.

interior (see discussion in Shomer et al., 2003). The effect of this is illustrated in Figure 5 for La^{3+}. If the charge is increased 10-fold to 0.211 M, the slope increases to 4.42 (top curve) from 1.46 for the standard run (middle curve)—a modest 3-fold increase. But if the microelectrodes and ζ-potential measurements failed to access the CW interior, then the PM is unlikely to do so either. Consequently, the slope of the upper curve in Figure 5 is almost certainly too steep even if the CW charge were 10-fold higher. In any case, $[La^{3+}]_{PM,CW}$ and $[La^{3+}]_{PM,medium}$ are well correlated.

What if the PM were less highly charged than the 0.307 μmol m^{-2} adopted for the Gouy-Chapman-Stern model (Table I)? This would appear to be unlikely because the value is in the midrange of several published values measured by more than one technique (surveyed in Yermiyahu et al., 1997). The smaller values for R_{Total} in Figure 3A indicate that ζ-potential measurements may not reflect all of the charge because (1) the ζ potential is the potential at the plane of shear near to but not at the PM surface, (2) the PMs of vesicles and protoplasts may not be entirely clean (some CW material and mucilage remaining), and (3) the PMs are rather hairy because of projecting polysaccharides and other structures that carry little charge but affect the electrophoretic dynamics of ζ-potential measurements. These features would have little effect upon the access of chemicals (dyes and radiotracers) used for measurements of σ_{PM}, nor would they affect the values of the binding constants that, in any case, agree remarkably well based upon two unrelated techniques of measurement (Kinraide et al., 1998). If $R_{Total} = 0.0307$ μmol m^{-2} rather than 0.307, then $[La^{3+}]_{PM,CW} = 6.5[La^{3+}]_{PM,medium}$ and $R^2 = 0.816$, meaning that the CW would have a greater

Figure 6. Selenium uptake by roots in response to $[SeO_4{}^{2-}]_{PM,CW}$ and $[SeO_4{}^{2-}]_{PM,medium}$. Other details are presented in Figure 2.

concentrating effect upon cations at the PM surface, but the concentrations would still be correlated with or without the influence of the CW. For divalent and monovalent cations, the concentrating effect would be less and the R^2 would be greater.

The bottom curve in Figure 5 illustrates the effect of changing another parameter. If the constant for Ca^{2+} binding to CW negative sites is increased from 0 to 1,000 M^{-1}, then the slope is reduced to from 1.46 to 1.12. Shomer et al. (2003) found that reversible cation binding (except for H$^+$) had trivial effects upon measured CW potentials in comparison to screening effects (which increase with ionic charge). I could find no reasonable adjustment of parameters that caused a reduction of slope to less than 1. Thus, the presence of a negatively charged CW always causes an increase in the concentration of cations at the PM surface if the PM is bathed in the CW Donnan phase rather than in the rooting medium directly. Is the CW ever positively

Figure 5. The effect of changing model parameters on the relationship of $[La^{3+}]_{PM,CW}$ to $[La^{3+}]_{PM,medium}$.

charged in any medium that allows growth? Apparently not, as discussed at length by Shomer et al. (2003). This is in contrast to the PM, which is often positively charged in acidic soil solutions.

APPLICATION OF THE MODELS TO SOME EXPERIMENTAL DATA

Figures 6 and 7 illustrate applications of the models to some published experimental data. In each case the generalities mentioned above are confirmed. Figure 6 is a continuation of Figure 2 and illustrates that selenium uptake corresponds to $[SeO_4^{2-}]_{PM,medium}$ and $[SeO_4^{2-}]_{PM,CW}$ about as well as to $\{SeO_4^{2-}\}_{PM,medium}$. Values for $[SeO_4^{2-}]_{PM,CW}$ are smaller than values for $[SeO_4^{2-}]_{PM,medium}$, as expected for an anion. Figure 7 illustrates that uptake of Ca^{2+} correlates better with $[Ca^{2+}]_{PM,medium}$ and $[Ca^{2+}]_{PM,CW}$ than with $[Ca^{2+}]_{medium}$. Values for $[Ca^{2+}]_{PM,CW}$ are larger than values for $[Ca^{2+}]_{PM,medium}$, as expected for a cation. The data for Figure 7 were obtained with outside-out PM vesicles so that CWs were not present. Figure 7C presents the PM-surface concentration of Ca^{2+} as though a CW were present and as though the PMs were bathed in the Donnan phase of the CWs.

The conclusion by Gage et al. (1985) that the "Enzymic removal of the yeast cell wall does not affect the kinetics of Rb^+ uptake by the cells, nor the inhibition of this uptake by Ca^{2+}" (p. 1) is compatible with the preceding analyses. This may be taken to mean that CWs and PMs fail to interact (diagrammed Fig. 1, top) or that multiple interactions counteract one another. To understand the latter, consider solutions numbers 1 and 5 in Tables II and III. Under the heading $[Na^+]_{PM,medium}$ (without interaction), we see that $[Na^+]$ at the PM surface is depressed 31% when $CaCl_2$ is increased from 0.1 to 1 mM because of the depolarizing effect of Ca^{2+}. The solution bathing the PM changes from 1 mM Na^+ and 0.1 mM Ca^{2+} to 1 mM Na^+ and 1 mM Ca^{2+}. Under the heading $[Na^+]_{PM,CW}$ (with interaction), we see that $[Na^+]$ at the PM surface is depressed 33% when $CaCl_2$ is increased from 0.1 to 1 mM. The solution bathing the PM changes from 7.07 mM Na^+ and 5.00 mM Ca^{2+} to 2.97 mM Na^+ and 8.80 mM Ca^{2+}.

ALTERNATIVE METHODS OF ANALYSIS

In the analyses above, I modeled CW-PM interaction as though the PM were bathed in the Donnan-phase solution of the CW. In reality, the PM may be pressed tightly against the CW so that the exterior PM surface may be substantially influenced by the Donnan phase but not bathed entirely in the Donnan-phase solution. Assume that the CW is a slab of permeable material with surface charges and binding sites. In this way we may treat the CW with a Gouy-Chapman-Stern model instead of the Donnan-plus-binding model. The model parameters are presented in Table I. Although Shomer
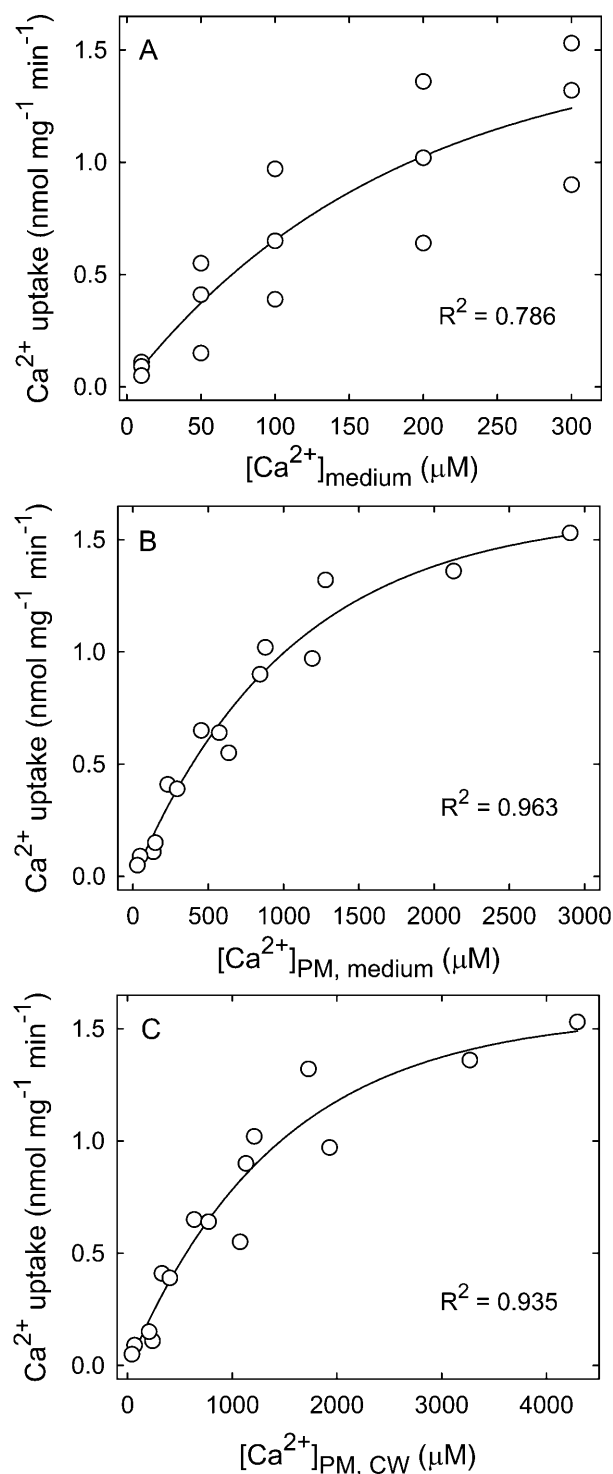
Figure 7. Ca^{2+} uptake plotted against Ca^{2+} concentrations in various phases. $[AlCl_3]$ in the external bathing medium was 0, 2, or 5 μM. Data are from Huang et al. (1996), who measured Ca^{2+} uptake into outside-out PM vesicles from wheat roots. E_m was clamped at -100 mV.
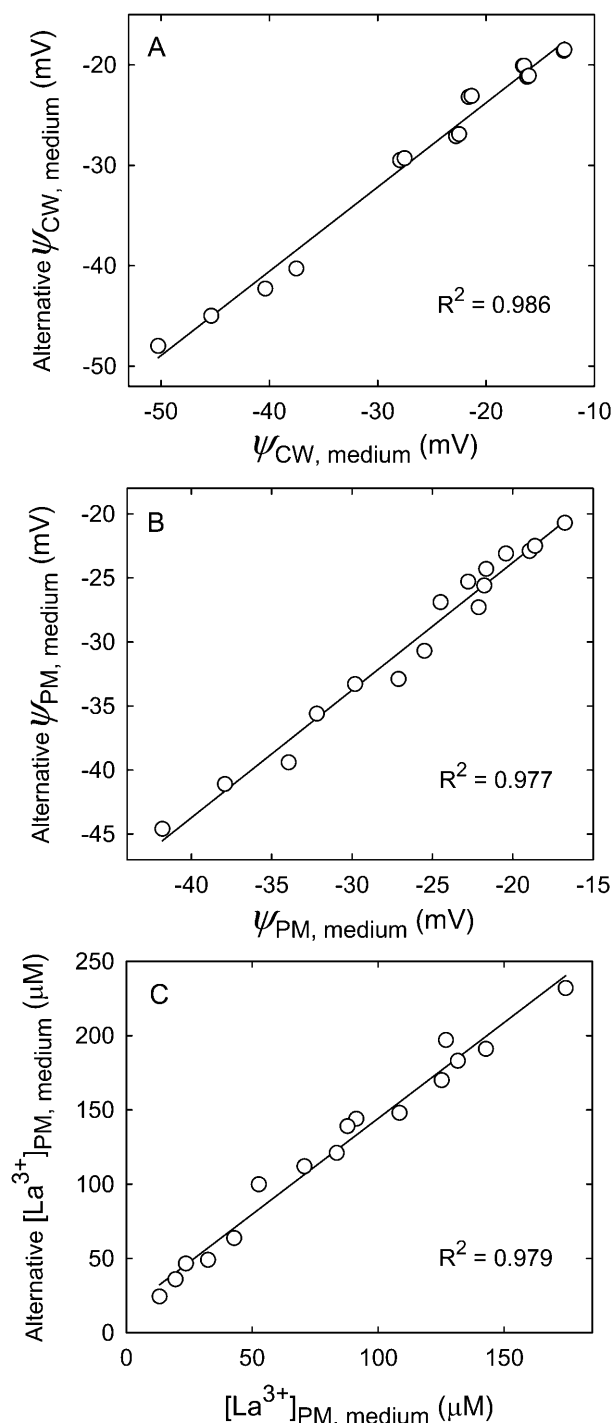
Figure 8. Electrical potentials and La^{3+} concentrations computed by an alternative model. Alternative $\psi_{CW,medium}$ refers to CW surface potential computed by a Donnan-plus-binding model. Alternative $\psi_{PM,medium}$ refers to the potential at the CW-PM interface, and Alternative $[La^{3+}]_{PM,medium}$ refers to concentrations at the CW-PM interface.

et al. (2003) considered a Donnan-plus-binding model (negative charges and binding sites distributed throughout the CW) more appropriate than a Gouy-Chapman-Stern model (sites restricted to the CW surface), the latter model generated values that correlated with measured potentials slightly better than did the former model (Fig. 3B).

The potentials computed by the Gouy-Chapman-Stern model for the CW surface (Alternative $\psi_{CW,medium}$) resemble closely the potentials computed by the Donnan-plus-binding model ($\psi_{CW,medium}$; Fig. 8A). For the extreme case of interaction between the CW and PM, imagine that the negative binding sites at the PM surface (R^- under the heading "G-C-S for PM" in Table I), the neutral binding sites at the PM surface (P^0 under the heading "G-C-S for PM" in Table I) and the negative binding sites at the CW surface (R^- under the heading "G-C-S for CW" in Table I) all lie in a plane—a plane in equilibrium with the rooting medium. A Gouy-Chapman-Stern model may be used to compute potentials at this CW-PM interface. Figure 8B indicates that those values (Alternative $\psi_{PM,medium}$) correlate well with the potentials of the PM bathed directly in the rooting medium ($\psi_{PM,medium}$). The effect is that the CW-PM interface attracts cations slightly more and anions slightly less than a naked PM (Fig. 8C).

CONCLUSION

According to these electrostatic analyses, the CW would appear to have only slight effects upon ion concentrations at the PM surface. Consequently, the electrical potentials of CWs and PM surfaces may be computed as though each were bathed directly in the rooting medium. These computed potentials correspond well to potentials that can be measured and may be used to compute free ion concentrations or activities in the respective phases. Computed ion activities at the PM surface correlate well with ion uptake, intoxication, and the alleviation of intoxication—responses that often correlate poorly with ion activities in the rooting medium.

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